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(57) Abstract

The invention provides a polypeptide consisting of 3 to 38 amino acid residues, having the sequence of SEG ID:1 or a portion thereof; and analogous polypeptide derivatives by virtue of point mutation, amino acid substitution, deletion or addition; SEQ ID:1 = $A^{1}PPP^{4}PE^{6}H^{7}R^{8}GGPEEF^{14}EGAGDG^{20}$ $EPP^{23}EDDDSATGLAFRTPN^{38}$ wherein the sequence includes histidine residue H^{7}; and wherein E^{6} may be substituted in order of preference by $D^{6} > T^{6}$; and wherein R^{8} may be substituted by A^{8}; which sequence is recognised by anti-gG2 positive human sera from patients with HSV-2 infection and is not recognised by anti-gG2 negative sera from patients with HSV-1 infection; and antibodies raised thereto; and prophylactic and therapeutic and diagnostic uses thereof relating to HSV-2 infection.

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IDENTIFICATION OF SHORT PEPTIDE SEQUENCES REPRESENTING EPITOPES OF GLYCOPROTEIN G OF HSV-2 USING A PHAGE PEPTIDE DISPLAY LIBRARY

Introduction

Identification of antigenic regions within viral proteins subserves a number of functions. Detailed knowledge of epitopes which induce protective immune responses may allow generation of prophylactic subunit vaccines; synthetically-derived antigenic epitopes may be used in diagnostic assays to detect virus-specific antibodies; mapping of epitopes within a whole protein may provide important clues as to the three-dimensional structure of that protein, and may enhance understanding of the mechanisms of immune escape adopted by the virus.

The recently described phage peptide display library technology (16,17) is a powerful tool for the identification of individual epitopes recognised by antibodies. Phage peptide libraries typically comprise more than 10^7 different phage clones, each expressing a different peptide, encoded in the single-stranded DNA genome as an insert in one of their coat proteins. Phage clones displaying peptides able to mimic the epitope recognised by a particular antibody are selected from the library by the antibody, and the sequences of the inserted peptides deduced from the DNA sequences of the phage clones. This approach has the major advantages that (i) no prior knowledge of the primary sequence of the target antigen is necessary, (ii) epitopes represented within the antigen either by a linear sequence of amino acids (linear epitope) or by the spatial juxtaposition of amino acids distant from each other within the primary

sequence (conformational epitope) are both identifiable, and (iii) peptidic mimotopes of epitopes derived from non-proteinaceous molecules such as lipids and carbohydrate moieties can also be generated (12, 14).

Herpes simplex virus type 2 (HSV-2) is the main cause of recurrent genital herpes (1). The vast majority of individuals infected with HSV-2, however, give no clinical history of disease, and yet these asymptomatic individuals will shed virus from epithelial surfaces at intervals, and are therefore an infection risk for their sexual partners (9,11). Establishment of serological assays which can distinguish between antibodies to HSV-1 and HSV-2 is difficult due to the considerable shared antigenicity of the two viruses. Nevertheless, such assays would find wide application in the development of rational programmes designed to reduce transmission of infection, eg to identify sexual partners who are discordant for HSV-2 infection as part of a strategy to reduce the incidence of neonatal herpes simplex infection, a disease of high morbidity and mortality (2, 4, 11).

The glycoprotein G (gG) molecule of HSV-2 has a large insert (over 500 amino acids) compared with its counterpart in HSV-1 (15), and has therefore attracted much attention as a likely source of type-specific antigens. Indeed, gG-2-based assays for the detection of HSV-2 antibodies using *Helix pomatia*-purified gG-2 as antigen in immunoblot and ELISA formats have been described (6, 7, 13). However, difficulties in large-scale production of gG2 of sufficient purity have precluded the widespread availability of such assays.

Following the work of McGeoch et al [15], WO 90/13652 (Triton Biosciences Inc.) disclosed proteins and polypeptides from the unique DNA sequence of about 1461 base pairs (coding for about 486 amino acids) of the HSV-2 envelope glycoprotein G gene, which sequence was not found in the HSV-1 gene. This was suggested to provide epitopic regions type specific for HSV-2 and not for HSV-1.

An alternative approach to the use of whole gG2 would be to construct an assay using synthetic peptides representing key gG2 epitopes as antigen. We describe the use of a phage library expressing random 15-mer peptides to identify a variety of peptide sequences recognised by 3 monoclonal anti-gG2 antibodies. Proof is provided that at least some of these peptides are also recognised by human sera known to contain anti-HSV-2 antibodies, thus validating this approach towards the development of a cheap and widely applicable assay for the detection of human anti-gG2 antibodies.

The present invention provides a polypeptide consisting of 3 to 38 amino acid residues, having the sequence of SEQ ID:1 or a portion thereof; and analogous polypeptide derivatives by virtue of point mutation, amino acid substitution, deletion or addition;

SEQ ID:1 $A^{1}PPP^{4}PE^{6}H^{7}R^{8}GGPEEF^{14}EGAGDG^{20}$
$EPP^{23}EDDDSATGLAFRTPN^{38}$

wherein the sequence includes histidine residue H^{7}; and wherein E^{6} may be substituted in order of preference by $D^{6}>T^{6}$; and wherein R^{8} may be substituted by A^{8}; which sequence is recognised by anti-gG2 positive human sera from

patients with HSV-2 infection and is not recognised by anti-gG2 negative sera from patients with HSV-1 infection.

The invention also provides a polypeptide which is antigenic.

The invention also provides a polypeptide which is immunogenic and is capable of inducing antibodies in an immunised host against type-specific HSV-2 gG.

The invention also provides pharmaceutical composition containing as an active ingredient an immunogenic polypeptide.

The invention also provides a vaccine composition containing as an active ingredient an immunogenic polypeptide together with a physiologically acceptable adjuvant and/or carrier and/or diluent.

The invention also provides an antibody to the polypeptide obtainable by immunisation of a host with the immunogenic polypeptide.

The invention also provides a recombinant DNA molecule comprising a DNA sequence encoding a polypeptide.

The invention also provides a filamentous bacteriophage including, in at least a proportion of its major coat protein sub-units, multiple display of a polypeptide.

The invention also provides a vaccine composition comprising a bacteriophage together with a physiologically acceptable adjuvant and/or carrier and/or diluent.

The invention also provides a substantially pure non-glycosylated polypeptide.

The invention also provides a method of testing for the presence of type-specific HSV-2 gG2 antibodies in a fluid, which comprises contacting the fluid with one or more polypeptide(s) and testing whether or not antibodies bind to the polypeptide(s).

The invention also provides a method of testing for the presence of type-specific HSV-2 gG2 antibodies in a fluid, which comprises contacting the fluid (i) with a labelled form of one or more polypeptide(s) and (ii) with antibodies, whereby antigen in the fluid competes with polypeptide(s) in binding to the antibodies.

The invention also provides a test kit for testing for the presence of HSV-2 type specific antibodies in a fluid, which comprises:
(i) a solid phase on which is immobilised one or more polypeptide(s); and
(ii) means for detecting binding of antibodies to polypeptide(s).

The invention also provides a test kit for testing for the presence of HSV-2 type specific antibodies in a fluid, which comprises:
(i) a solid phase on which is immobilised one or more polypeptide(s) in labelled form;
(ii) antibodies; and
(iii) means for detecting competitive binding of antibodies to polypeptide(s).

The invention also provides a method of diagnosis of HSV-2 infection which comprises employing the test method.

The invention also provides a method of diagnosis of HSV-2 infection which comprises employing the test kit in the test method.

The invention also provides a method of treatment of HSV-2 infection which comprises administration to an infected patient of an immunologically therapeutically effective amount of the vaccine composition.

The invention also provides a method of treatment of HSV-2 infection which comprises administration to an infected patient of an immunologically therapeutically effective amount of the antibody.

The invention also provides a method of prevention of HSV-2 infection which comprises administration to a patient a prophylactically effective amount of the vaccine composition.

The invention also provides a method of prevention of HSV-2 infection which comprises administration to a patient a prophylactically effective amount of the antibody.

The invention also provides a polypeptide which is the sequence SEQ ID:2 consisting of A^1 to G^{20} of SEQ ID:1 (PT71).

The invention also provides a polypeptide which is the sequence SEQ ID:3 consisting of P^4 to P^{23} of SEQ ID:1 (PT 487).

The invention also provides a polypeptide conjugated to a Biotin-NH_2 terminus by a lysine (K) group.

The invention also provides a polypeptide which is the sequence SEQ ID:42 consisting of A^1 to F^{14} to SEQ ID: 1 (PT444).

Surprisingly, the sequence SEQ ID:1 according to the invention corresponds to a region downstream of that targeted in WO 90/13652, this region having some HSV-2 unique portions but also some homology to HSV-1 portions. Despite this HSV-1 homology polypeptides from this region and antibodies raised thereto can exhibit good HSV-2 type specificity. The selection of the claimed region of the native sequence to provide polypeptides, and their serological and antigenic specificity, is unexpected in view of the teaching of the prior art towards using unique HSV-2 regions. It is particularly interesting and surprising that truncated versions of SEQ ID: 1 which are shorter than SEQ ID:2 (PT71), such as SEQ ID:42. (PT444), by virtue of omitting a downstream portion having homology with HSV-1 are able to show type-specific HSV-2 activity which appears to be less HSV-2-specific than the longer version (PT71) which includes a region having HSV-1 homology. On the basis of prior art teaching one might expect the inclusion of parts of a sequence not unique to HSV-2 might lead to a reduction in the ability to distinguish between HSV-2 and HSV-1 in a diagnostic test.

From the foregoing, PT71 SEQ ID:2 is currently the most preferred polypeptide for use in a diagnostic, by virtue of its considerable ability to distinguish HSV-2 from HSV-1.

The invention is described with reference to the accompanying drawings in which:

Figure 1 shows

a) Inhibition of binding of H5 to gG2 by phage clones

Two phage clones (2.10 ◆ and 3.15 ■) selected by mAb H5 are able to inhibit binding of H5 to gG2; no inhibition is seen with wild-type phage (M13 ▲).

b) Inhibition of binding of E5 to gG2 by phage clones

Phage clone (12.18 ■) selected by mAb E5 is able to inhibit binding of E5 to gG2, while no inhibition is seen with wild-type phage (M13 ▲). Inhibition by phage clone 12.17 (◆) is weak at the concentrations shown here, but at higher phage concentrations, inhibition of up to 70% was achieved.

c) Inhibition of binding of F11 to gG2 by phage clones

Two phage clones (8.22 ◆ and 9.4 ■) selected by mAb F11 are able to inhibit binding of F11 to gG2; no inhibition is seen with wild-type phage (M13 ▲).

Figure 2 shows

a) Inhibition of binding of H5 to gG2 by synthetic peptides

Peptides Ch16685 (●) and PT73 (▲), with sequences derived from phage clone inserts 2.10 and 3.15 respectively, and PT71(◆), with sequence derived from gG2, were able to inhibit binding of H5 to gG2 at all concentrations tested, but inhibition was seen with PT72 (■), a scrambled version of PT71 only at 500ug/ml. Reduced inhibition was seen with peptide PT74 (X), in which amino acids derived from pVIII at the N-terminal side of the insert were omitted, compared

with PT73 (▲). However, PT75(□) which lacked the PFT motif present in PT73 was still able to inhibit. The 8mer peptide PT156 (O), a shortened version of PT71 was also able to inhibit binding of H5 to gG2. Details of the sequences of these peptides are given in Table 2.

b) Inhibition of binding of E5 to gG2 by synthetic peptides

Peptides Ch16688 (◆) and Ch16689 (■), with sequences derived from phage clone inserts 12.18 and 12.17 respectively and PT71 (▲), with sequence derived from gG2, were able to inhibit binding of E5 to gG2 , but no such inhibition was seen with PT166 (O). Details of the sequences of these peptides are given in Table 2.

c) Inhibition of binding of F11 to gG2 by synthetic peptides

Peptide Ch16687 (■) with sequence derived from phage clone insert 8.22 and PT173(▲), with sequence derived from gG2, were able to inhibit binding of F11 to gG2. No inhibition was seen using Ch16686 (◆), with sequence derived from phage clone insert 9.4. Details of the sequences of these peptides are given in Table 2.

Figure 3 shows

Reactivity of human sera with peptides

The reactivity of human sera with four different peptides is illustrated :PT71, derived from gG2 native sequence containing epitopes recognised by mAbs H5 and E5 (Fig. 3a), PT75, derived from phage insert selected by mAb H5 (Fig. 3b), Ch16687 derived from phage insert selected by mAb F11 (Fig. 3c), and PT173, derived from gG2 native sequence, containing epitope recognised by mAb F11 (Fig. 3d). The sera were used at a dilution of 1:25 and fall into 4 groups based on the presence of antibodies to HSV-1 and HSV-2

proteins detectable by Western Blotting: 1) antibodies to neither HSV-1 nor HSV-2; 2) antibodies to HSV-1 only; 3) antibodies to HSV-2 only; 4) antibodies to both HSV-1 and HSV-2.

Figure 4 shows 92 human sera were tested for their reactivity with both PT71 and gG2. The results are shown as a graph of reactivity with PT71 against reactivity with gG2. There was a correlation coefficient of 0.61 using a Pearson correlation test, giving a probability of $p<0.0001$ that these results correlate by chance.

Figure 5 shows the results of an experiment in which 10 positive and 5 negative sera were used to stain the peptides whose sequences are given in the table, which had been synthesised on membrane. Positive sera are defined here as sera which are reactive with gG2 in ELISA and were taken from patients who were culture positive for HSV-2 at the time the serum sample was taken; negative sera are those which do not react with gG2 in ELISA and were taken from patients who were culture positive for HSV-1 at the time the serum sample was taken.

Figure 6 shows graphs of reactivity with biotinylated peptides SEQ ID:2 (PT71) and SEQ ID:3 (PT487), and a control peptide SEQ ID:44 (PT482). The test used a panel of " positive" and " negative" sera in ELISA against streptavidin-coated plates, with or without the biotinylated peptide attached. The results are shown as OD with buffer alone or with peptide. Thus, one is looking not at the magnitude of the OD alone, but at the difference in OD when the peptide is added to the plate. This therefore takes into account any reactivity with streptavidin alone. SEQ ID:44

is PT482 and has the sequence PPEHRGGPEEFEGAGDGEPP-K-Biotin-NH_2.

Figure 7 shows Western blot results of four experiments showing the ability of polypeptides of the invention (PT71 and PT444) to distinguish between HSV-1 and HSV-2 positive, +/-, -/+ and negative serum samples, as compared to a gG2 fragment (PT 445) and Gg2.

PT444: SEQ ID:42: $A^{1}PPPPEHRGGPEEF^{14}$

PT445: SEQ ID:43: KTPPTTPAPTTPPPTSTHAT

Materials and Methods

Monoclonal antibodies

Anti-gG2 monoclonal antibodies (mAbs), O2E10.A3.H5, O1B9.E5, P4A10.F11 (abbreviated to H5, E5 and F11 respectively throughout), in the form of culture supernatants were used. All mAbs are positive against gG2 in ELISA. H5 was used at a dilution of 1:100, and E5, F11 at a dilution of 1:200, as this was found to be optimal in ELISA against gG2.

Phage peptide display library

The library used was a gift from Dr. G. Smith (Missouri, USA) containing approximately 10^{8} different phage clones based on the filamentous phage fd-tet which is composed of the genome of the filamentous phage fd and a segment of the transposon Tn10, coding for tetracycline resistance, thus allowing the selection of infected host bacteria by plating out in the presence of tetracycline. In addition to a wild-type gene VIII encoding the major coat protein pVIII, the

phage in this library were engineered to express a recombinant form of gene VIII containing a degenerate DNA insert encoding random 15-mer peptides (Smith, personal communication) and are, therefore, type 88 vectors (18). The recombinant gene VIII is under the control of a *tac* promoter; the ratio of the peptide-displaying to wild-type pVIII can, therefore, be altered by varying the concentration of iso-propyl-thio-galactose (IPTG) added to the host bacterial culture.

Bacteria

The K91Kan strain of *E. coli*, a λ-derivative of K-38 was used throughout. It is Hfr Cavalli and has chromosomal genotype *thi*. Bacteria were cultured in LB medium (Sigma), with the addition of kanamycin (50 g/ml), tetracycline (20 g/ml) or IPTG (1mM) where appropriate.

Infection of bacteria

Infections were carried out by incubating phage for 30 mins at room temperature (RT) with an equal volume of K91Kan, grown to log-phase in LB containing kanamycin. LB containing an inducer tetracycline concentration of 1 g/ml was added and the bacteria were incubated for a further 45 minutes at 37°C.

Preparation of polyethylene glycol (PEG)-precipitated phage

Phage were purified from the culture supernatants of infected bacteria by addition of 1/5th of the volume of 20% PEG/2.5M NaCl, followed by incubation for 1hr at 4°C. The precipitated phage were pelleted, resuspended in Tris-

buffered saline (TBS), and the PEG precipitation was repeated. Phage from a culture supernatant volume of 5ml were usually resuspended in a final volume of 150 l of TBS. The optical density was then read at 269nm and the concentration of the phage preparations were standardised to 150ug/ml, assuming that an O.D. of 1 is equivalent to a concentration of 3.8mg/ml.

Biopanning

Three rounds of biopanning were carried out with each mAb. During the first round, ELISA wells (Nunc Maxisorp) were used as the solid phase; they were coated with aliquots of mAb over night at RT in a humid atmosphere, washed in TBS, blocked in TBS-1% BSA, then washed in TBS-0.05% BSA. One aliquot of the library containing 10^{10} phage in 50 l TBS-0.05% BSA was added to the antibody-coated well, for 1hr at RT. Unbound phage were removed and the wells were washed 4 times in TBS-0.05% BSA and 4 times in TBS. 50 l of elution buffer (0.2M glycine, 0.1M HCl, 0.1% BSA, 0.1mg/ml phenol red, pH 2.2) were added for 10-20 seconds, then removed and neutralised by addition of Tris-HCl pH8.8 (Sigma T5753). The phage eluted from each antibody were used to infect log phase K91Kan, then grown over night in LB containing tetracycline. They were purified by PEG-precipitation.

The second and third rounds of biopanning were carried out using a 20 l aliquot of Goat anti-mouse coated dynabeads (Dynal) as the solid phase. The beads were washed 4 times in TBS, incubated with a 50 l aliquot of the mAb, then washed and blocked. During round 2, a 50 l aliquot of the PEG precipitated phage from round 1 was incubated with the mAb-coated beads, then washed. Bound phage were eluted,

amplified and purified by PEG-precipitation as in round 1. During round 3, PEG-precipitated phage from round 2 were used. Again, bound phage were eluted, amplified and purified by PEG-precipitation. Phage eluted during the third round of biopanning were used to infect bacteria which were then plated out at a low concentration on LB-agar tetracycline plates to allow individual phage clones to be isolated.

ELISA to identify positive phage clones

ELISA wells (Nunc Maxisorp) were coated by incubating overnight with Rabbit anti-fd antibodies (Sigma) diluted 1:1000 in coating buffer (carbonate-bicarbonate buffer, pH 9.6). After each incubation the wells were washed with PBS-0.05% Tween 20. The plates were blocked by addition of PBS-0.05% Tween 20-1% BSA (blocking buffer). Individual phage clones were grown overnight in LB containing tetracycline and IPTG, to maximise expression of the recombinant form of gene VIII containing the peptide insert. The rabbit anti-fd coated wells were incubated in turn for 1 hour at RT with supernatant from such cultures, the test mAb diluted in blocking buffer (dilutions as described above) and alkaline phosphate conjugated Goat anti-mouse IgG (Sigma A1682) diluted to 1:1000 in blocking buffer. pNPP at 1mg/ml in diethanolamine buffer (10% diethanolamine, pH 9.8, 0.5mM $MgCl_2$, 0.02% sodium azide) was used as a substrate for the alkaline phosphatase and the O.D. of each well was read at 405nm.

Sequencing

ssDNA was prepared from 1.5ml overnight cultures by PEG precipitation followed by phenol-chloroform extraction and ethanol precipitation. Sequencing was carried out using a Sequenase Version 2.0 T7 DNA polymerase kit (Amersham) according to the manufacturer s instructions. The oligonucleotide AGCAGAAGCCTGAAGAGAGTC (SEQ ID: 4), complementary to the genomic DNA of the phage 3' of the insert, was used as a primer.

Peptides

Peptides were a gift from Peptide Therapeutics Ltd. (Cambridge, UK). They were synthesised by standard f-moc methodology.

Inhibition ELISAs

Wells were coated with *Helix Pomatia* lectin-purified gG2 at a dilution of 1:500 in coating buffer. After blocking, peptides or phage were added simultaneously with the mAb diluted in blocking buffer. The mAbs were diluted by a factor of 1:2 compared with the concentration used in the ELISA above. Binding of the mAb was detected using the same procedure as in the ELISA above.

gG2 and Peptide ELISAs

ELISA wells (Nunc Maxisorp) were coated by incubating over night with peptides at 5ug/ml in PBS. After each incubation the wells were washed with PBS-0.05% Tween 20. The plates were blocked by addition of a 1:10 dilution of Boehringer Mannheim ECL blocking solution (Cat. No. 1500 694) in PBS. Incubation buffer was a 1:20 dilution of this reagent in

PBS. Wells were incubated in turn with serum diluted 1:25 and horse radish peroxidase conjugated Rabbit $F(ab)_2$ anti-human IgG (Dako P0406) diluted to 1:1000 in PBS-10%NGS. Sigma Fast OPD tablets (Sigma P9187) were used as a substrate for the peroxidase and the O.D. of each well was read at 490nm after stopping the reaction with 2M H_2SO_4.

Human sera

24 patient sera were collected at the Virology Department, Centre for Infectious diseases and Microbiology, Westmead Hospital, Sydney, Australia. These had previously been characterised by Western Blotting (7) for the presence of IgG reactive with HSV-1 and HSV-2 proteins, and fall into 4 groups of 6 sera based on those reactivities : no antibodies to either HSV-1 or -2 (group 1; antibodies to HSV-1 only (group 2); antibodies to HSV-2 only (group 3); antibodies to both HSV-1 and -2 (group 4).

Results

Selection of phage clones. 3 mAbs (H5, E5, F11) with specificity for gG2 were used to screen the library of phage containing random 15-mer peptide inserts. After three rounds of biopanning, individual phage clones were isolated and screened by ELISA to identify those which bound strongly to the antibody of interest, and those which gave a clear positive signal were sequenced. The sequences of the phage clone inserts are given in Table 1. Note that SEQ ID:14 " native" is PT71; the same as claimed in claim 21 SEQ ID:2.

Identification of motifs amongst the sequences of the phage clone inserts and within the native sequence of gG2. Motifs could be identified amongst the phage clones for mAbs H5, E5, and F11 using Clustal W (1.4) for Multi Sequence Alignment (http://biology.ncsa.uiuc.edu/BW/BW.cgi), followed by minor manual adjustment. For mAb H5, it can be seen that the motif ([D/E]HRS) tended to appear at the N-terminal side of the 15-mer insert. We postulated that adjacent amino acids derived from the natural protein VIII sequence may have contributed to the antibody binding site, and therefore have included these amino acids (PAE) in the alignment. The sequence of gG2 was then scanned using Clustal W - Multi Sequence Alignment program (http://biology.ncsa.uiuc.edu/BW.BW.cgi) to identify regions with sequence similarity to these motifs (native sequence, Table 1).

Inhibition of binding of the mAbs to gG2 by phage clones. If the inserts present in phage clones selected by the mAbs truly contained epitopes or mimotopes of the native antigen, then such clones should inhibit binding of the relevant mAb to gG2. To test this hypothesis, two representative phage clones for each mAb were used in an inhibition assay. Each phage clone was used at a range of concentrations. For each mAb, wild-type phage M13 was used as a negative control to ensure that inhibition of binding of the mAb to gG2 was due to the phage insert rather than the mere physical presence of the phage. The percentage inhibition, compared with wells to which no phage were added, was calculated. The results are shown in Figure 1(a-c). For each mAb, both phage clones tested were able to inhibit binding of the mAb to gG2 although the degree of inhibition varied for different clones. Inhibition of E5 by 12.17 was particularly low at

the range of concentrations shown in Fig. 1b, but when it was used at higher concentrations, up to 2.5mg/ml, inhibition of as much as 70% was observed. In comparison, little inhibition was observed using the wild-type phage M13 over the same range of concentrations.

Inhibition of binding of the mAbs to gG2 by peptides representing phage inserts or the primary amino acid sequence of gG2. Further proof that the epitopes of gG2 recognised by each of the mAbs were indeed represented by the phage clone inserts was sought by testing a number of synthetic peptides for their ability to inhibit binding of the mAbs to gG2. The sequences of the peptides used are given in Table 2. For mAbs H5, F11 and E5, two peptides, with sequences derived from the inserts of phage selected by that mAb, and one peptide derived from the native sequence of gG2 with most similarity to the motif common to phage selected by the mAb (native sequence, table 1) were tested. At least one irrelevant peptide was included in each assay as a negative control.

For mAb H5 three further peptides were used : (i) PT74, to test the hypothesis that phage amino acids at the N-terminal side of the insert were contributing to the antibody-binding site, (ii) PT75, to investigate the importance of a second motif (PFT) apparently common to some of the phage selected by this antibody, though not selected by ClustalW as a motif, and (iii) PT156, to localise the sequence of importance within gG2.

Peptides were added at a range of concentrations from 500µg/ml to 7.5 µg/ml. The percentage inhibition, compared with wells to which no peptide was added, was calculated.

Binding of mAb H5 to gG2 was inhibited by both peptides PT73 and Ch16685 with sequences derived from phage clone inserts 3.15 and 2.10 respectively, and by the peptide PT71 derived from the sequence of gG2 (Fig. 2a). The inhibition of binding of H5 to gG2 was clearly dependent on the sequence of the peptides as PT72, a scrambled version PT71, did not have this effect. In phage clone 3.15, from which the sequence of PT73 was derived, the amino acids at the N-terminal side of the insert were clearly necessary for the formation of the epitope recognised by H5; binding of H5 to gG2 was not inhibited by peptide PT74 which was identical to PT73 except that instead of the 5 phage amino acids (PAEGD) at the N-terminal side of the insert, 5 phage amino acids (MLSFA) from the C terminal were added. However, this was not the case for phage clone 2.10 as a peptide with sequence derived from its insert only (Ch16685) was able to inhibit H5 binding as effectively as PT73. Another apparent motif, PFT, common to a number of the phage clones selected by H5 was not essential as these amino acids could be deleted, as in peptide PT75, without preventing the peptide's ability to inhibit binding of H5 to gG2 (Fig. 2a). The region of gG2 which is involved in binding H5 was further localised by the use of peptide PT156, an 8mer peptide derived from PT71, which was also able to inhibit binding of H5 to gG2 (Fig. 2a).

Similarly, binding of E5 to gG2 could be inhibited by both of the peptides Ch16688 and Ch16689, derived from phage 12.18 and 12.17 respectively, and by PT71, derived from native gG2 sequence (Fig. 2b); binding of F11 to gG2 could be inhibited by the peptide Ch16687, derived from phage

8.17, and PT173, derived from native gG2 but not by Ch16686 derived from phage 9.4 (Fig. 2c).

Cross-inhibition of mAbs by peptides. The above experiments indicated that peptides with sequences derived from the insert of phage clones selected by a particular mAb were able to inhibit binding of that mAb to native gG2. Similarly, peptides with sequences derived from the known primary sequence of gG2 were also able to inhibit H5, E5, and F11 binding to gG2. All of these inhibitory peptides were then tested at a single concentration (250ug/ml) for their ability to inhibit binding of the other mAbs to gG2 (Table 3).

With one exception, peptide sequences selected by one mAb did not inhibit binding of heterologous mAbs to gG2, a result to be expected if the 3 mAbs did indeed recognise separate epitopes within gG2. The exception was peptide Ch16689, derived from phage clone 12.17 selected by mAb E5. This peptide also inhibited H5, though not F11. Peptide PT71, which inhibited both H5 and E5 has sequence derived from gG2 and contains the motif recognised by both mAbs.

Binding of human sera to peptides. When the peptides were bound directly to wells of an ELISA plate only peptides PT71, PT75, Ch16687 and PT173 were reactive with their associated mAbs (results not shown). A panel of 24 human sera, whose anti-HSV-1 and -2 reactivity had previously been determined by Western blotting were tested for their ability to bind to these peptides. The results are illustrated in Fig. 3. The binding of all sera lacking any anti-HSV reactivity (group 1) or containing only anti-HSV-1 antibodies (group 2) to all 4 peptides was very low. In

contrast 9/12 sera known to contain anti-HSV-2 antibodies were reactive with PT71 (Fig. 3a), 7/12 with Ch16687 (Fig. 3c) and 8/12 with PT173 (Fig. 3d). Clearly, sera from group 3 (with both anti-HSV-1 and -2 reactivity) showed the greatest reactivity with these peptides. None of the sera were reactive with PT75.

Discussion

Using the phage library technology, we have identified peptides which are able to mimic 3 epitopes of gG2. The epitopes are defined by 3 mAbs, H5, E5, F11 which were used to select phage from a library of approximately 10^8 different phage expressing random 15mer peptides as a part of the major coat protein. A number of filamentous phage libraries expressing random peptides have been described, varying in terms of the size of the peptide insert, the coat protein used to display the peptide, and in the presence or absence of constraints on the flexibility of the inserted peptides (5). Each library has its particular advantages and disadvantages. We chose to use an unconstrained 15-mer library expressed in protein VIII. The increased length of this insert may allow development of internal secondary structure, so increasing the possibility that the insert, when synthesised as an isolated peptide, will adopt the same conformation as the inserted peptide. A potential disadvantage of this effect is that any secondary structure within the insert could impair recognition of a sequence motif common to selected phage clones, as the relevant amino acid residues within the inserts mediating binding to antibody will not necessarily be contiguous in the insert sequences. However, as our primary aim was not to identify the specific amino acid - antibody contact residues, but

rather to identify peptide sequences capable of binding to anti-gG2 monoclonal antibodies, this was deemed not to be a problem.

Positive phage clones recognised by each mAb were identified by ELISA and assayed for their ability to inhibit binding of the relevant mAb to gG2 to verify that the interaction between the mAb and phage was occurring through the antigen-specific domain of the antibody. One would expect a given test mAb to select multiple phage clones whose inserts are structurally similar to each other, and to the epitope against which the mAb was raised. Comparison of the amino acid sequences of the inserts of a number of selected phage clones may, therefore, lead to recognition of a motif of commonly recurring residues. This information can then be used to scan the native sequence of the target antigen (if known) in order to determine whether the motif is present in a linear format within that sequence. Such an analysis of the sequences of positive phage clones for three of the mAbs revealed common motifs, different for each mAb, suggesting that they recognise distinct epitopes. That the mAbs recognise distinct epitopes is further supported by the fact that none of the phage identified by any individual mAb was recognised in ELISA by any of the other mAbs (data not shown), and that, in general, the mAbs were not inhibited by peptides associated with other mAbs.

The first epitope is defined by mAb H5. A motif common to the majority of the phage clones selected by this mAb (EHRSP) could be identified within the native gG2 sequence, and two synthetic peptides containing this sequence (PT71, PT156), one only 8 amino acids long, as well as peptides with the sequence of two phage clone inserts (PT73, PT75,

Ch16685), could inhibit binding of H5 to gG2. Amino acids from outside the 15mer insert were found to contribute to the epitope in at least one of the phage clones (3.15) recognised by this mAb, as a peptide in which these amino acids were not included (PT74) was unable to inhibit binding of H5 to gG2. That these amino acids were important in a number of the phage clones selected by H5 was suggested by the fact that the motif common to the majority of the clones was usually found at the N-terminal end of the insert. However, these amino acids did not appear to be essential in the case of the phage clone 2.10, as a peptide synthesised with the sequence of its insert alone (Ch16685) was able to mimic the epitope in the inhibition ELISA. The insert of one phage clone (2.4) recognised by H5 had a sequence apparently unrelated to that of the remaining clones, even though it was consistently positive in the ELISA with H5 but not with an irrelevant antibody, nor with F11 or E5. This may, therefore, be a mimotope which is able to mimic the shape and charge distribution of the native epitope (3).

The epitope defined by E5 is apparently adjacent to that defined by H5 since the motif common to phage clones selected by E5 is found in the region of gG2 present in peptide PT71. However, this is a distinct motif as neither PT73 or Ch16685, nor PT156, a shortened version of PT71, inhibit binding of E5 to gG2. Interestingly, Ch16689 a peptide with the sequence of the insert of one of the phage clones selected by E5 did inhibit binding of H5, as well as E5, to gG2, and this peptide has a region (EHP) with sequence similarity to the motif of clones selected by H5. However, the E5 peptide Ch16688 which has the three amino acids EHR does not bind to H5, and Ch16689 also inhibits

binding of H7 to gG2, so it is not clear at present whether the interactions of Ch16689 with H5 and H7 are specific.

The epitope defined by F11 comes from a different region of gG2. A shorter motif (TPL) was found to be common to phage clones selected by this mAb and a region of gG2 including amino acids 359 - 378 containing this motif (PT173), as well as two peptides with the sequence of phage clones selected by F11 (Ch16686, Ch16687), inhibited binding of F11 to gG2.

Thus, via use of the phage peptide display library technique, we have successfully defined a number of peptides (PT71, PT73, PT173, PT156, Ch16685, Ch16688, Ch16689, Ch16687, PT173) capable of binding to HSV type-specific monoclonal antibodies. These peptides therefore act as representations of the epitopes seen by those mAbs within native gG2. Their precise secondary structures may indeed be exact replicas of the native epitopes such that the mAbs bind to exactly identical amino acids within the peptides as within gG2. Alternatively, the peptides may be true mimotopes, adopting the shape and charge characteristics of the epitope, but being composed of dissimilar residues. The value of having identified these peptides lies in their potential use as antigens capable of distinguishing between anti-gG1 and anti-gG2 antibodies.

The gG2 epitopes we have described were defined by murine mAbs. In order to determine whether these epitopes are also antigenic in humans infected with HSV-2, it was necessary to bind the peptide mimics to the solid phase in an ELISA. However, whilst the majority of the peptides tested were able to inhibit binding of their associated mAbs to gG2, only a subset of these peptides (PT71, PT75, Ch16687 and

PT173) retained reactivity with their cognate mAb when bound to the solid phase. Presumably, in solution, the peptides are free to adopt an appropriate conformation which will allow reactivity with the mAb but when bound to the solid phase, their conformation is restricted and the epitope may be lost.

Those peptides which retained their antigenicity were tested in ELISA for their reactivity with a well-characterised panel of human sera. Each of the peptides PT71, Ch16687 and PT173 showed reactivity with some of those sera which were known to react with HSV-2 proteins in Western blots, but with none of the sera which were reactive with HSV-1 proteins only or with neither HSV-1 nor HSV-2. Interestingly, for each of these peptides the strongest reactivity was seen with sera containing both anti-HSV-1 and -2 antibodies. It is likely, given the the epidemiology of HSV-1 and HSV-2 infections, that this group of patients had been exposed to HSV-1 first, followed by HSV-2, and therefore, it is interesting to speculate that the stronger reactivity in this group against type 2 specific epitopes may be a result of the generation of type-common T helper cells during the first infection.

In addition, 3/6 of the sera with only anti-HSV-2 antibodies showed clear reactivity against PT71. It is not surprising that not all these sera react with a single peptide. The pattern of recognition of multiple epitopes within a large and complex protein such as gG2 by different individuals is likely to be heterogeneous. Some sera in this group were also reactive with peptides representing F11 epitopes, although the reactivity with these peptides was less impressive. The data presented in Fig. 3 confirm the type-

specificity of the epitopes we have described, and also indicate that these epitopes are recognised by the human immune system. This raises the possibility of generating a peptide-based assay for the detection of HSV-2 type-specific antibody in human sera which may find wide clinical application (2, 4, 11).

Presentation of Peptides

For diagnostic use it may be advantageous to label the peptide, for example, with biotin using amino-hexanoic acid biotin incorporated during synthesis, or using N-hydroxysuccinimido biotin to derivatise free amino groups (such as the N-terminus), or any lysyl side chains). It may also be convenient to use other labelling reagents such as acridinium esters or europium chelates which are used in a number of commercial assay systems. Radioactive labelling might also be useful, e.g. by the appending of a tyrosine residue to the C- or N-terminus of the peptide to allow introduction of iodine atoms via oxidation of ^{125}I iodide ion in the presence of chloramine-T according to widely used methods for radioimmunoassay. Similarly radioactive iodine could be incorporated via the Bolton-Hunter reagent (an N-hydroxysuccinnimide ester) according to methods described in the Amersham catalogue. Tritium would also be a convenient label - incorporated during synthesis with one or more radioactive amino acid, or post-synthetically using amino-directed reagents such as tritiated N-succinimidyl propionate (Amersham catalogue).

For solid phase assays such as ELISA, it may also be advantageous to increase the valency of the antigenic

peptide by coupling it, for example to branched lysine cores according to methods described by James Tam of Rockerfeller University. This could also be achieved via attachment of the peptide to poly-L or poly-D lysine (or poly-L or poly-D glutamic acid, or these polymers with aspartic acid in place of glutamic) using homo to heterobifunctional cross-linking agents such as glutaraldehyde or carbodiimides, according to methods described in the Pierce (Rockford Illinois) Chemical Company catalogue. Amino-acid copolymers containing an abundance of any of the three residues individually or in combination (Asp, Glu, Lys) or analogues of these residues containing carboxylate or amino acid side chains (e.g. ornithine in place of Lys) might also be used. Such polymers could be of random or ordered sequence, and might usefully contain other amino acids such as alanine, beta alanine, epsilon amino caproic acid or glycine as spacers to facilitate the optimal degree of substitution of the peptide without contributing spurious additional epitopes to the construct. In particular the randomness of the sequence of the amino acid copolymer core would contrive to avoid the generation of spurious antigenic reactions with human sera, since the abundance of any individual motif generated in the random copolymer would be effectively diluted among numerous other random sequences. Antigenically irrelevant carrier proteins (e.g. human serum albumin) could also be used for the purpose of increasing the valency of the antigenic peptide, using similar cross-linking chemistries.

In solid phase assays it may also be advantageous to attach the peptide indirectly to the solid phase to minimise adverse effects of direct adsorption to the solid phase, which might include adverse influence on accessibility of the peptide, or critical subgroups (e.g. side chains)

therein. Such adverse effects might also include conformational effects e.g. interference by the solid phase in the attainment of antigenically relevant conformations by the peptide. Any of the methods described above to increase the valency of the peptide could also be used to facilitate the attachment of the peptide to the solid phase in a solid phase assay.

Carriers (other than phage) might also be used with any of the peptides to generate immunogenic constructs capable of eliciting antibodies or cellular (e.g. T-cell) immune responses against the peptides and against HSV-2. Such carriers would most advantageously be non-human in origin - thereby enhancing the ability of the human immuno system to response to the peptides (e.g. by providing a carrier function such as T-cell epitopes). Exemplary carriers would be tetanus and diphtheria toxoids, hepatitis-B virus cores, keyhole limpet haemocyanin, virus particles (such as bacteriophage). Carriers might also be synthetic - such as poly-L lysine, poly D-lysine, branched lysine (multiple antigenic peptide constructs referred to above) etc. Carriers might also comprise synthetic peptides (e.g. collinearly synthesised with HSV-2 peptides) comprising known or candidate T-cell epitopes of HSV-2 or any other pathogen or molecule.

The peptides may also be used to purify antibodies from infected sera for the purpose of standardisation of the diagnostic test or for the purpose of passive immunotherapy of infected individuals.

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Table 1 **Sequences of the inserts of phage clones recognised by mAbs and native sequence with homology to the phage clones**

mAb	Phage clone	Sequence of insert	[SEQ ID : No]
H5	3.15	T S P F T P V I G P L E H R S (P A E)	5
	3.19	S T T N T P L V S H L E H R S (P A E)	6
	3.21	T G S V Y S P T G L L E H R S (P A E)	7
	2.5	R E T K L P F N V Y T E H R S (P A E)	x
	2.11	X P P F T S A V G G V D H R S (P A E)	9
	3.13	A P P F T S A V G G V D H R S (P A E)	10
	2.10	M D D D T E R F P T H R S L P	11
	3.9	R A R D H S S T A Q Q E H A T	12
	2.4	R A M A G R C R L L L S I G S	13
	Native	$_{551}$A P P P P E H R G G P E E F E G A G D G $_{570}$	14
E5	12.17	A T S L P P T E H P N M Y Q G	15
	12.21	A G G Y S P T E H A F H S P P	16
	12.28	T S T P T E H T Y P L E I I T	17
	12.16	D P G T E H A G V P L R H S A	18
	12.20	Y G A R P P E H L L Y S R A S	19
	12.18	S P L P E P P P P E H R A L V P	20
	12.4	Y M Q P D P P P P P L H A P D Y	21
	12.3	T R M P L P N H Y E P P P R T	22

Table 1 (contd.)

			[SEQ ID: No.]
F11	8.17	A L S S Q G G M S P E P T P L	23
	8.22	A L S S Q G G M S P E P T P L	24
	8.10	V S S R P T T H Y Y L P E P L	25
	8.13	T P E S T P L L P P F P R S V	26
	8.14	S T N P E P L P F P A E E L S	27
	8.16	Q K Y A P E T T P V S Y L G A	28
	9.1	H V L S S R P T T L A L P L F	29
	9.4	D Y T P Q T S L E L P P E S F	30
	9.5	T P A Q A Y P A L R S L I P W	31
	9.3	T A T T V T P R R T P Y A P I	32
	Native	$_{159}$P E K T P L P V S A T A M A P S V D P S$_{178}$	33

For each mAb, the sequences of the inserts of phage clones selected by that mAb are given, using the standard single letter amino acid code. For the clones selected by H5, the 3 amino acids at the N-terminal side of the insert are also shown, in brackets, for the sequences ending with (D/E) HRS. For mAbs H5, F11 and E5, the sequences were aligned using Clustal W (1.4) for Multi Sequence Alignment (http://biology.ncsa.uiuc.edu/BW/BW.cgi), followed by minor manual adjustment. The motifs found by alignment were rerun in Clustal W against the gG2 sequence to identify the native sequence most similar to the motif.

Table 2 Sequences of peptides

mAb	Peptide	Sequence
H5	Ch 16685	MDDDTERFPTHRSLP (phage 2.10 insert) SEQ ID : N. 11
	PT73	TSPFTPVIGPLEHRSPAEGD (phage 3.15 insert with amino acids derived from pVIII at the N-terminal side of the insert)
	PT71	APPPPEHRGGPEEFEGAGDG (gG2, amino acids 551 - 570) SEQ ID:N° 14 SEQ ID : No 34
	PT72	RAGPEGPPGEPGEADFEPGH (scrambled version of PT71) - negative control SEQ ID:N° 35
	PT74	MLSFATSPFTPVIGPLEHRS (phage 3.15 insert with amino acids derived from pVIII at the C-terminal side of the insert)
	PT75	MLSFAPVIGPLEHRSPAEGD (phage 3.15 insert without PFT motif) SEQ ID : N° 37 SEQ ID:N° 36
	PT156	EHRGGPEE (gG2, amino acids 556 - 562, 8mer variant of PT71) SEQ ID : No 38
E5	Ch16688	SPLPEPPPEHRALVP (phage12.18 insert) SEQ ID : N° 20
	Ch16689	ATSLPPTEHPNMYQG (phage 12.17 insert) SEQ ID : N° 15
	PT71	APPPPEHRGGPEEFEGAGDG (gG2, amino acids 551 - 570) SEQ ID : N° 14
	PT166	RMARPTEDVGVLPPHWAPGA - negative control SEQ ID : N° 39
F11	Ch16686	DYTPQTSLELPPESF (phage 9.4 insert) SEQ ID:N° 30
	Ch16687	ALSSQGGMSPEPTPL (phage 8.22 insert) SEQ. ID : N° 24
	PT173	PEKTPLPVSATAMAPSVDPS (gG2, amino acids 359 - 378) SEQ ID : N° 33

The sequences of the peptides used are given, using the standard single-letter code for amino acids, from the C terminus to the N terminus. The derivation of the sequence is give in brackets.

Table 3 Cross-inhibition of mAbs by peptides representing other epitopes. Peptides were all used at a single concentration of 250ug/ml

		Percentage inhibition of binding of mAb to gG2 by peptide			
Peptide	mAb associated with peptide	H5	E5	F11	H7
PT73	H5	77	0	0	0
Ch16685	H5	77	0	0	0
PT156	H5	67	0	0	0
PT71	H5 - E5	67	73	0	0
Ch16688	E5	0	80	0	0
Ch16689	E5	83	77	0	43
Ch16686	F11	0	0	0	0
Ch16687	F11	0	0	74	0
PT173	F11	0	0	84	0
Ch17783	H7	0	0	0	67

Table 4 Sequences of the inserts of the phage clones used as immunogens

Phage clone	Sequence of insert	
2.10	M D D D T E R F P T H R S L P	SEQ ID: No11
2.11	X P P F T S A V G G V D H R S	SEQ ID: No9
3.19	S T T N T P L V S H L E H R S	SEQ ID: No6
Native (SEQ ID:14)	...A P P P P E H R G G P E E F E G A G D G...	

Table 5 Number of mice surviving 14 days after intraperitoneal challenge with HSV-2

Immunising dose of phage (μg)	Unabsorbed	Polymixin B absorbed	Total
100	3 / 3	3 / 3	6 / 6
75	3 / 3	3 / 3	6 / 6
50	1 / 3	2 / 3	3 / 6
10	1 / 3	1 / 3	2 / 6

Claims

1. A polypeptide consisting of 3 to 38 amino acid residues, having the sequence of SEQ ID:1 or a portion thereof;
and analogous polypeptide derivatives by virtue of point mutation, amino acid substitution, deletion or addition;

SEQ ID:1 $A^{1}PPP^{4}PE^{6}H^{7}R^{8}GGPEEF^{14}EGAGDG^{20}$
$EPP^{23}EDDDSATGLAFRTPN^{38}$

wherein the sequence includes histidine residue H^{7};
and wherein E^{6} may be substituted in order of preference by $D^{6}>T^{6}$;
and wherein R^{8} may be substituted by A^{8};
which sequence is recognised by anti-gG2 positive human sera from patients with HSV-2 infection and is not recognised by anti-gG2 negative sera from patients with HSV-1 infection.

2. A polypeptide according to claim 1 which is antigenic.

3. A polypeptide according to claim 2 which is immunogenic and is capable of inducing antibodies in an immunised host against type-specific HSV-2 gG.

4. A pharmaceutical composition containing as an active ingredient an immunogenic polypeptide according to claim 3.

5. A vaccine composition containing as an active ingredient an immunogenic polypeptide according to claim 3 together with a physiologically acceptable adjuvant and/or carrier and/or diluent.

6. An antibody to the polypeptide of claim 1 obtainable by immunisation of a host with the immunogenic polypeptide of claim 3.

7. A recombinant DNA molecule comprising a DNA sequence encoding a polypeptide according to claim 1.

8. A filamentous bacteriophage including, in at least a proportion of its major coat protein sub-units, multiple display of a polypeptide according to claim 1.

9. A vaccine composition comprising a bacteriophage according to claim 8 together with a physiologically acceptable adjuvant and/or carrier and/or diluent.

10. A substantially pure non-glycosylated polypeptide according to claim 1, 2 or 3.

11. A method of testing for the presence of type-specific HSV-2 gG2 antibodies in a fluid, which comprises contacting the fluid with one or more polypeptide(s) according to claim 1, 2, 3 or 10 and testing whether or not antibodies bind to the polypeptide(s).

12. A method of testing for the presence of type-specific HSV-2 gG2 antibodies in a fluid, which comprises contacting the fluid (i) with a labelled form of one or more polypeptide(s) according to claim 1, 2, 3 or 10 and (ii) with antibodies according to claim 6, whereby antigen in the fluid competes with polypeptide(s) in binding to the antibodies.

13. A test kit for testing for the presence of HSV-2 type specific antibodies in a fluid, which comprises:
(i) a solid phase on which is immobilised one or more polypeptide(s) according to claim 1, 2, 3 or 10; and
(ii) means for detecting binding of antibodies to polypeptide(s).

14. A test kit for testing for the presence of HSV-2 type specific antibodies in a fluid, which comprises:
(i) a solid phase on which is immobilised one or more polypeptide(s) according to claim 1, 2, 3 or 10 in labelled form;
(ii) antibodies according to claim 6; and
(iii) means for detecting competitive binding of antibodies to polypeptide(s).

15. A method of diagnosis of HSV-2 infection which comprises employing the test method of claim 11 or 12.

16. A method of diagnosis of HSV-2 infection which comprises employing the test kit of claim 13 or 14 in the test method of claim 11 or 12.

17. A method of treatment of HSV-2 infection which comprises administration to an infected patient of an immunologically therapeutically effective amount of a vaccine composition according to claim 5 or 9.

18. A method of treatment of HSV-2 infection which comprises administration to an infected patient of an immunologically therapeutically effective amount of an antibody according to claim 6.

19. A method of prevention of HSV-2 infection which comprises administration to a patient a prophylactically effective amount of a vaccine composition according to claim 5 or 9.

20. A method of prevention of HSV-2 infection which comprises administration to a patient a prophylactically effective amount of an antibody according to claim 6.

21. A polypeptide which is the sequence SEQ ID:2 consisting of A^{1} to G^{20} of SEQ ID:1 (PT71).

22. A polypeptide which is the sequence SEQ ID:3 consisting of P^{1} to P^{23} of SEQ ID:1.

23. A polypeptide according to claims 1 to 3, 10, 21 or 22 conjugated to a Biotin-NH_2 terminus by a lysine (K) group.

24. A polypeptide which is the sequence SEQ ID:42 consisting of A^{1} to F^{14} to SEQ ID: 1 (PT444).

FIG 1

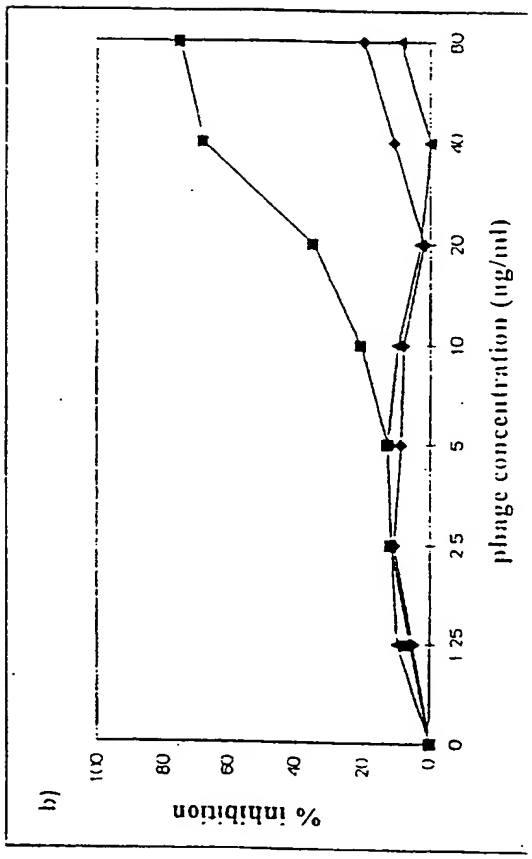

b)
% inhibition
100
80
60
40
20
0
0
1.25
2.5
5
10
20
40
80
phage concentration (ng/ml)

FIG 2

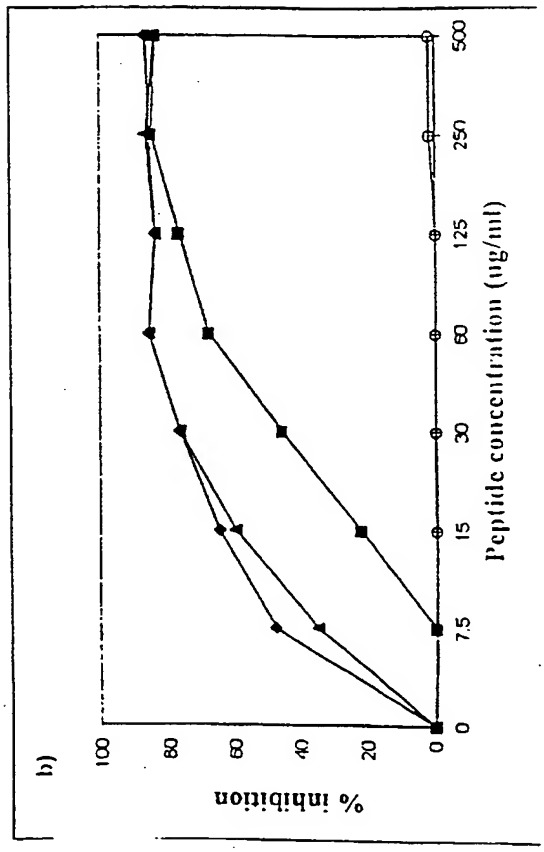
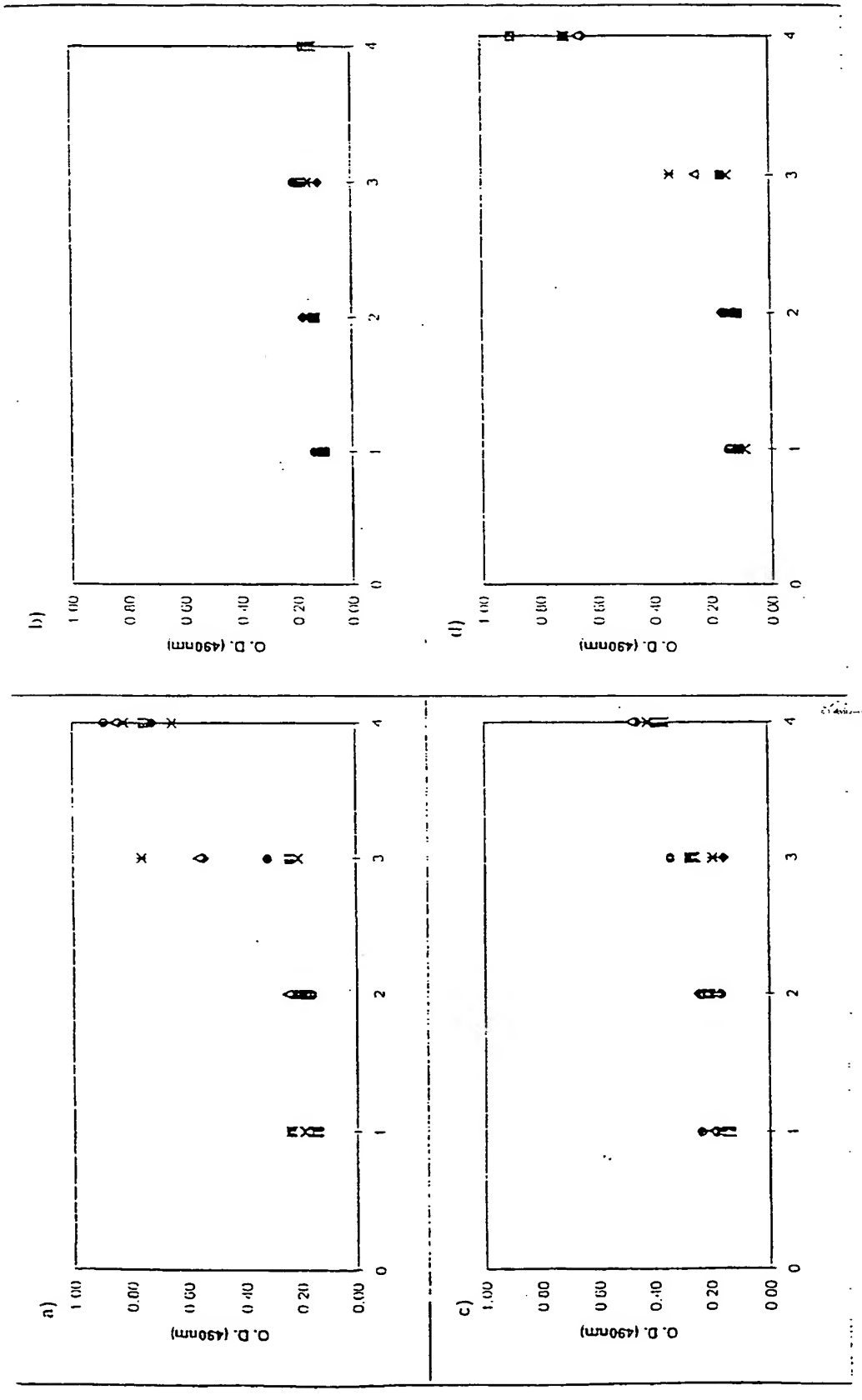

% inhibition
100
80
60
40
20
0
Peptide concentration (ug/ml)
0
7.5
15
30
60
125
250
500

FIG 3



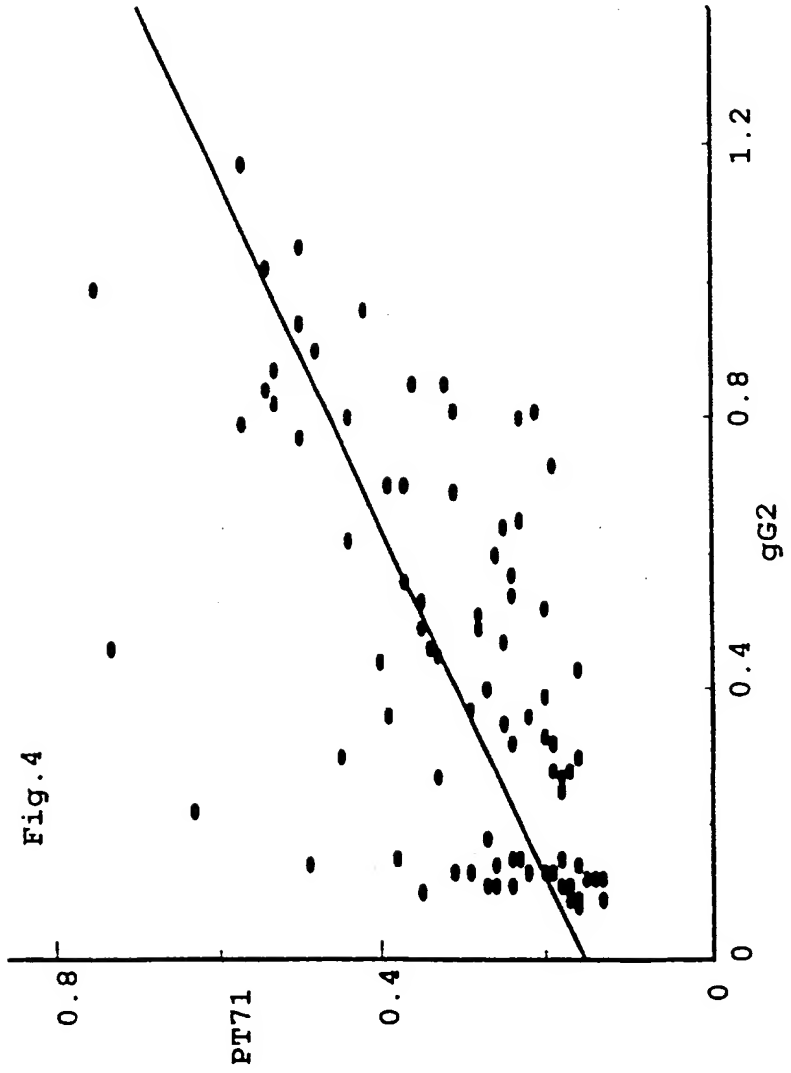


Fig.4

PEPTIDE	SEQ ID: No
APPPPEHRGGPEEFEGAGDG	2
PPPEHRGGPEEFEGAGDGEP	40
PEHRGGPEEFEGAGDGEPPE	41
HRGGPEEFEGAGDGEPPEDD	42
GGPEEFEGAGDGEPPEDDDS	43
PEEFEGAGDGEPPEDDDSAT	44
EFEGAGDGEPPEDDDSATGL	45
EGAGDGEPPEDDDSATGLAF	46
AGDGEPPEDDDSATGLAFRT	47
DGEPPEDDDSATGLAFRTPN	48

Figure 5a

	HSV2 serum														
	Positives										Negatives				
SEQ ID: No	1	2	3	5	7	8	14	26	41	42	10	11	19	29	43
2		+/-	+	+	+	+/-		+	+	+					
44	+/-	+/-	+	+	+	+	+/-	+	+	+	+/-				
41	+	+	+	+	+	+	+	+	+	+			+/-		
42	+	+	+	+	+	+	+	+	+	+			+/-		
43	+	+	+	+	+	+	+	+	+	+	+/-		+/-	+	+/-
44	+	+	+	+	+	+	+	+	+	+				+	
45	+	+	+	+	+	+	+	+	+	+				+	+/-
46	+	+	+	+	+	+	+	+	+	+					
47	+	+	+	+	+	+	+	+	+	+				+/-	+/-
48	+	+	+	+	+/-		+		+	+		+/-			

Fig 5b

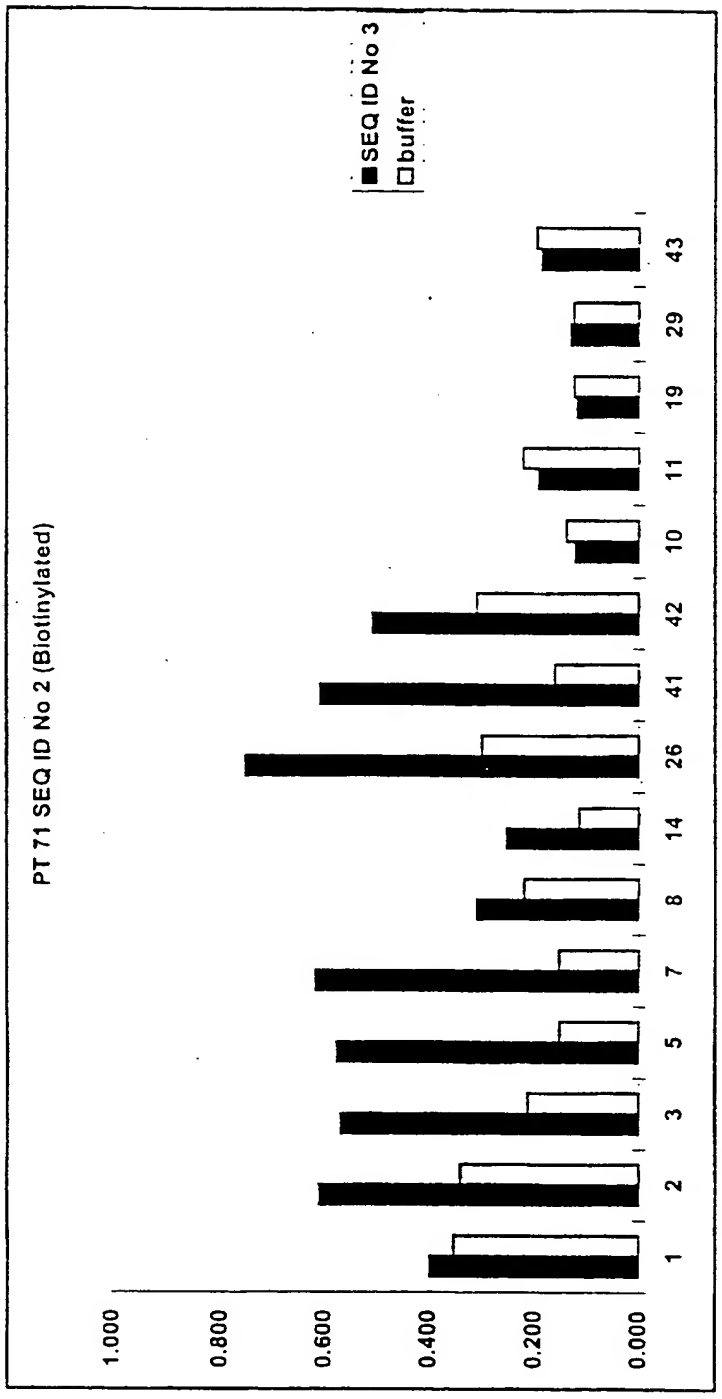

PT 71 SEQ ID No 2 (Biotinylated)
SEQ ID No 3
buffer
1.000
0.800
0.600
0.400
0.200
0.000
1
2
3
5
7
8
14
26
41
42
10
11
19
29
43

Fig 6a

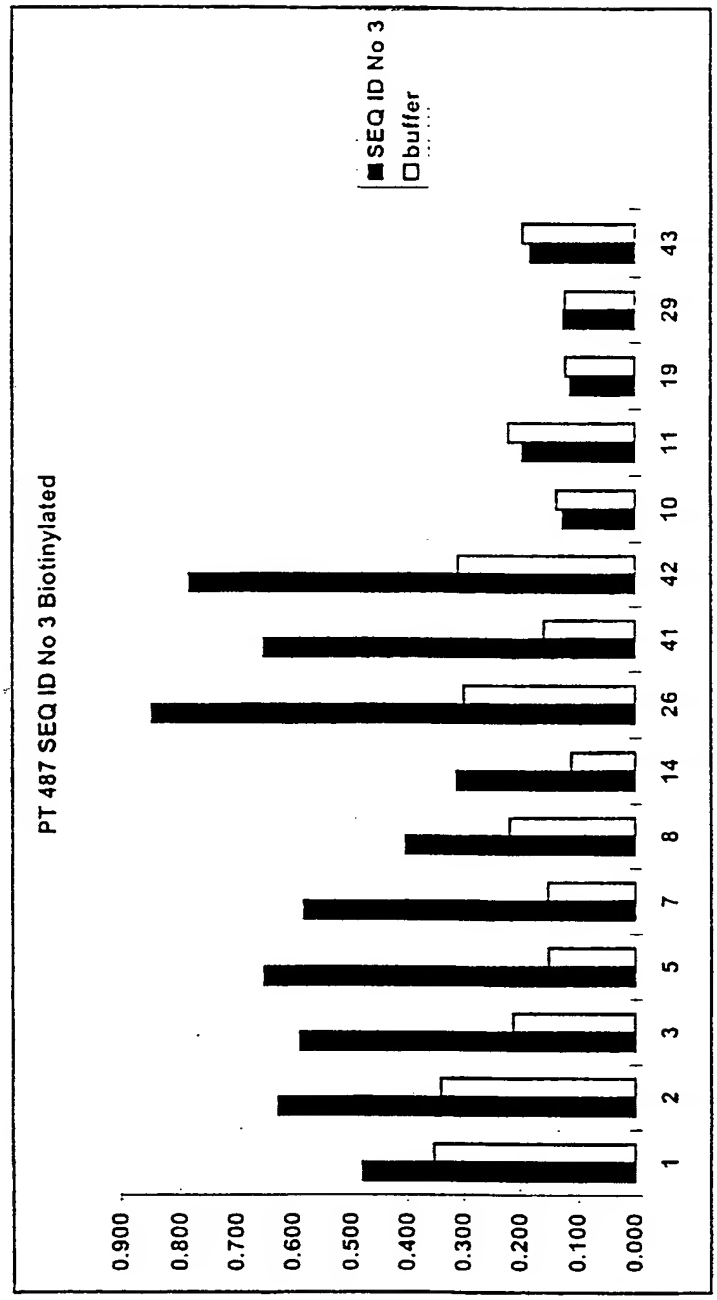

PT 487 SEQ ID No 3 Biotinylated
SEQ ID No 3
buffer
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
1
2
3
5
7
8
14
26
41
42
10
11
19
29
43

Fig 6b

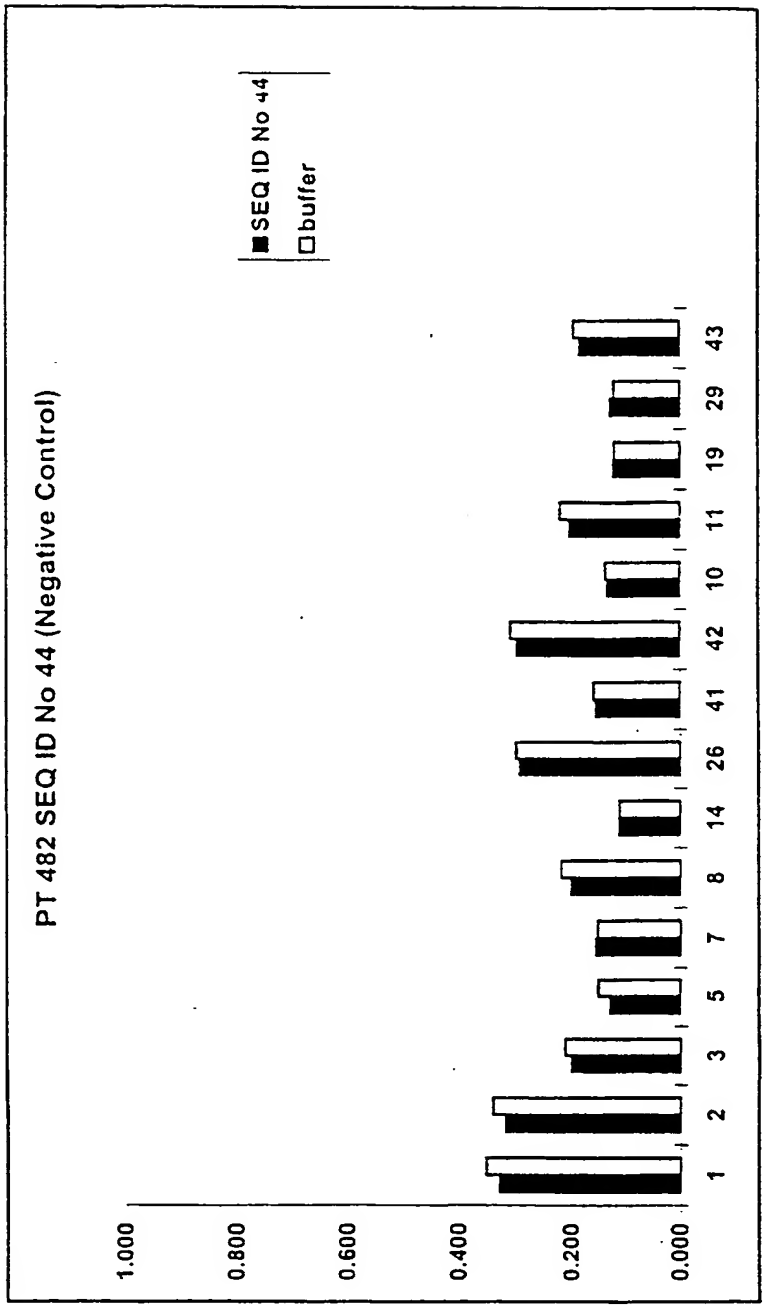

PT 482 SEQ ID No 44 (Negative Control)
SEQ ID No 44
buffer
1.000
0.800
0.600
0.400
0.200
0.000
1
2
3
5
7
8
14
26
41
42
10
11
19
29
43

Fig 6c

SEQ ID: No 2

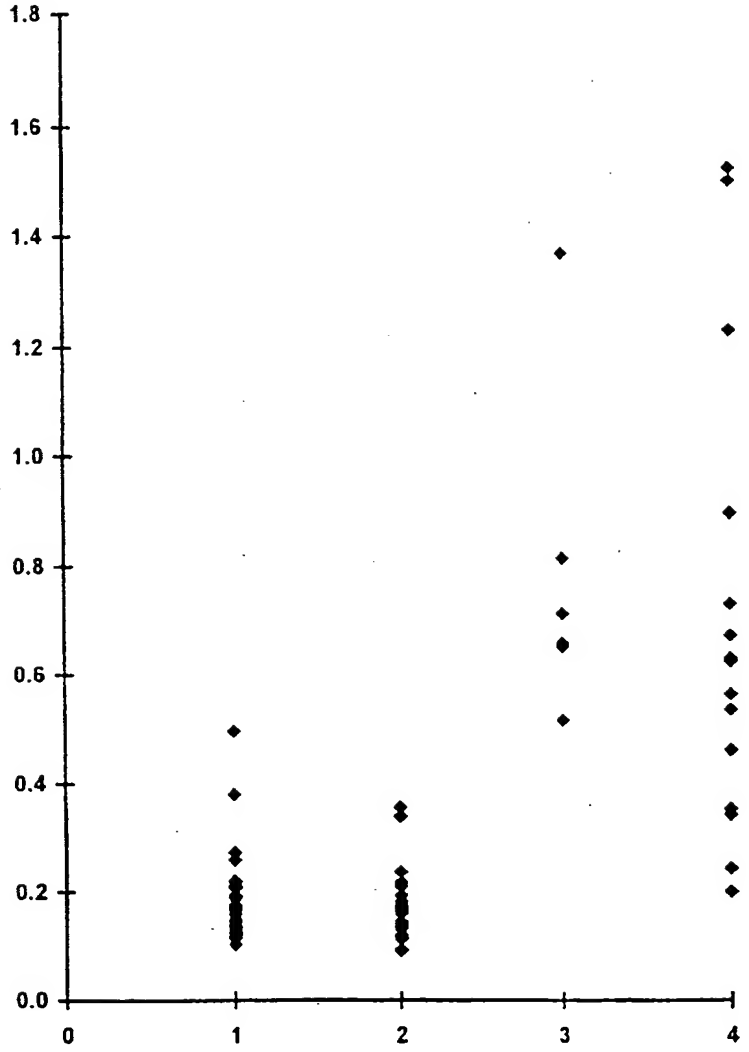


Fig 7a

SEQ ID: No 42

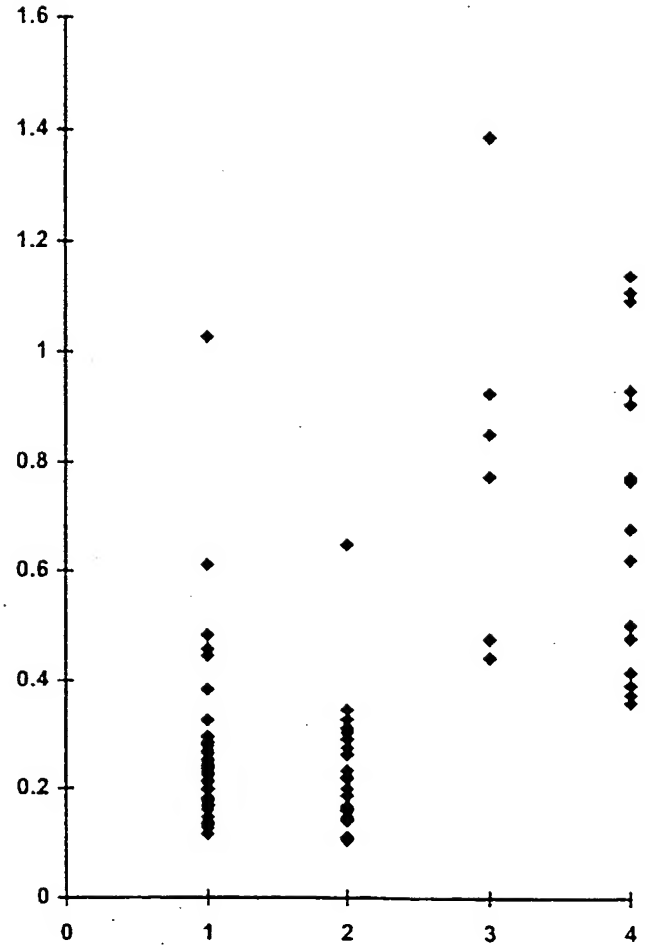


Fig 7b

gG2 Native Sequence

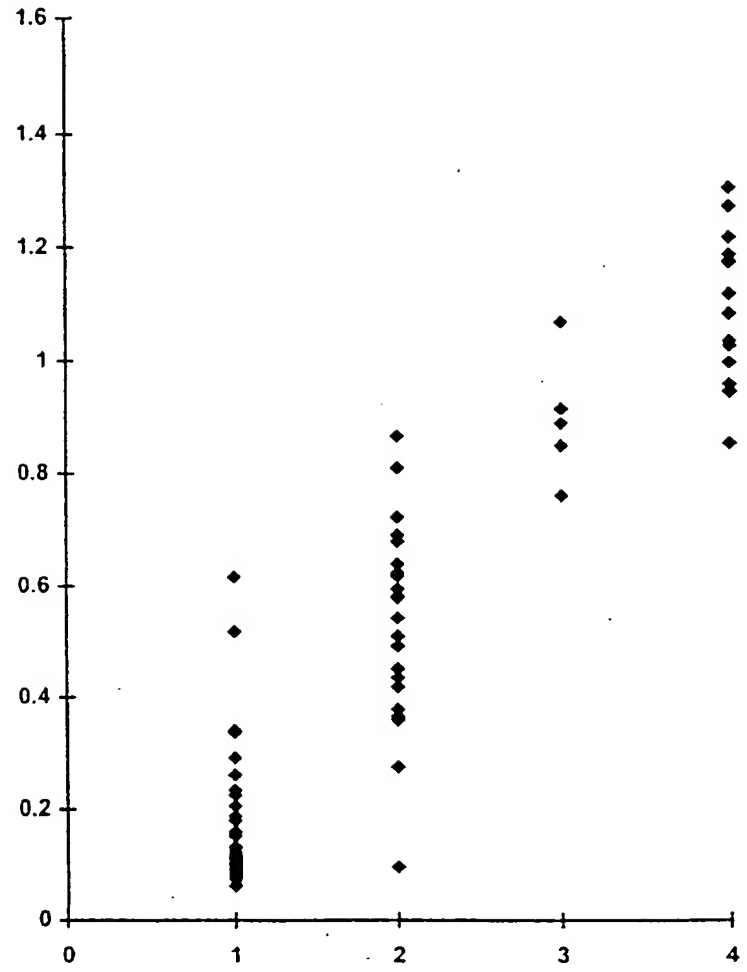


Fig 7c

SEQ ID: No 43

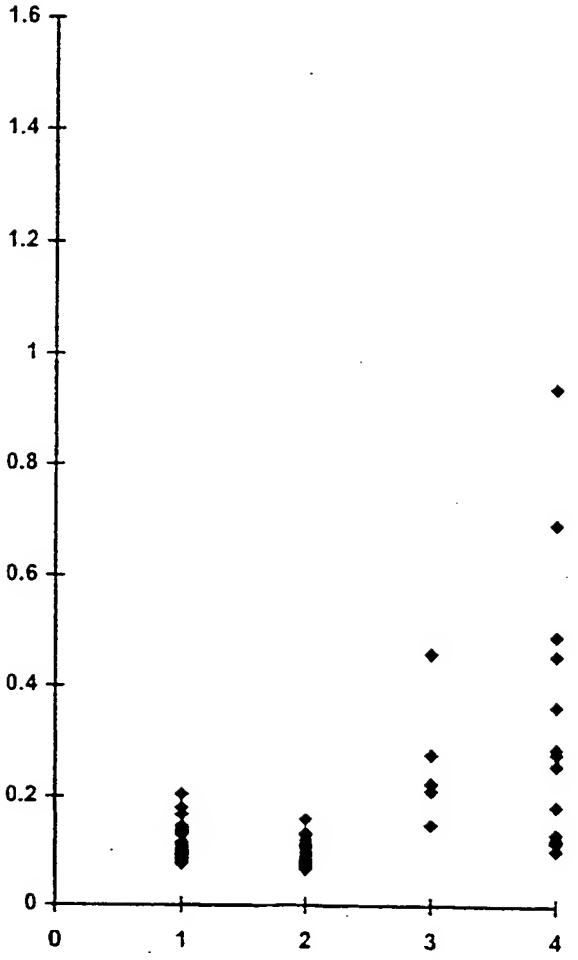


Fig 7d

INTERNATIONAL SEARCH REPORT

Intern .al Application No
PCT/GB 97/01990

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/035 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. LEVI ET AL.: "Peptide sequences of glycoprotein G-2 discriminate between herpes simplex virus type 2 (HSV-2) and HSV-1 antibodies" CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, vol. 3, no. 3, May 1996, pages 265-269, XP002042984	1-6, 10-12, 15-20
Y	see page 266, column 1, paragraph 2; figure 1 see page 267, column 2, paragraph 4 ---	7,13,14
Y	WO 90 13652 A (TRITON BIOSCIENCES INC) 15 November 1990 see abstract; claims 20-25,45,48,51 see page 32, paragraph 1 see page 11 - page 12 ---	7,13,14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1997	11. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 97/01990

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 17443 A (DU PONT) 14 November 1991 see the whole document see claim 3	11-16
A	CHEMICAL ABSTRACTS, vol. 106, no. 17, 1987 Columbus, Ohio, US; abstract no. 132537, page 145; column 2; XP002042985 cited in the application see abstract & MCGEOCH ET AL.: "DNA sequence and genetic content of the HindIII l region in the short unique component of the herpes simplex virus type 2 genome" J. GEN. VIROL., vol. 68, no. 1, 1987, pages 19-28,	
A	GRIHALDE N D ET AL: "Epitope mapping of anti-HIV and anti-HCV monoclonal antibodies and characterization of epitope mimics using a filamentous phage peptide library" GENE, vol. 166, no. 2, page 187-195 XP004043072	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ational application No.
PCT/GB 97/01990

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97 01990

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of ----------the following reasons:

Claims Nos.: 11-12,15-20

---

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

Claims Nos.: 1

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

A very large number of compounds falls under the scope of claim 1, therefore the Search Division judged not economically reasonable to draw a search report covering the entire subject of this claim. The search was restricted to SEQ 1 and to the 24 pentadecapeptide fragments obtained by scanning the 39 residues of the sequence SEQ 1 with 15mer peptides.

Remark : Although claims 17-20 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the composition.
Remark : Although claims 11, 12, 15 and 16 are directed to a diagnostic method practised at least in part on the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/GB 97/01990

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9013652 A	15-11-90	EP 0471778 A	26-02-92
		US 5470704 A	28-11-95
		US 5656457 A	12-08-97
		US 5665537 A	09-09-97
WO 9117443 A	14-11-91	EP 0527200 A	17-02-93